Patterns of multi-drug resistant bacteria at first culture from patients admitted to a third level University hospital in Calabria from 2011 to 2014: implications for empirical therapy and infection control

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Surveillance of antimicrobial drug resistance is fundamental to guide empirical treatment. However, the European Antimicrobial Resistance Surveillance Network provides a general picture, which might not be applicable to clinical settings that are excluded from this survey. We evaluated resistance patterns of ESKAPE isolates over a four-year period in a third level University hospital in the province of Catanzaro (Southern Italy). In this retrospective study, we evaluated the frequency of ESKAPE isolates with different resistance patterns (group 1=low-resistant bacteria; group 2=multi-drug and extremely drug-resistant bacteria; group 3=pan-resistant bacteria), stratified by year (2011, 2012, 2013 and 2014), hospital units (intensive care units, medical and surgical units) and by sample type (urine, blood, wound swabs, respiratory samples, other samples). $\chi^2$ test was applied to find differences between isolates with different resistance patterns by hospital unit and by organs and systems. Cochran-Armitage trend test was applied to assess the trend in resistance patterns during the four years analyzed. Amongst 2385 isolates, *Escherichia coli* (38%) was the most frequent, followed by *Pseudomonas aeruginosa* (15%), *Klebsiella pneumoniae* (14%), *Staphylococcus aureus* (13%), *Acinetobacter baumannii* (9%), *Enterococcus faecalis* (8%) and *Enterococcus faecium* (3%). From 2011 to 2014, frequency of isolates in group 2 plus 3 decreased from 23% to 14% ($\chi^2=55.093; p<0.0001$), particularly for *E. coli* and *K. pneumoniae*, but the trend increased for *S. aureus* (from 5% in 2011 to 10% in 2014), and remained stable for the other species. Frequency of isolates in group 2 plus 3 was higher in intensive care units for *K. pneumoniae* ($\chi^2=32.292; p<0.0001$), *A. baumannii* ($\chi^2=6.947; p<0.0001$) and *S. aureus* ($\chi^2=22.079; p<0.0001$). It was also higher from blood than from different sources for most species. Our results provided indications for an empirical antimicrobial choice. Importantly, antibiotic resistances have declined in recent years, probably due to optimized infection control and therapeutic algorithms, but still remain a significant problem. In particular, *A. baumannii* and *K. pneumoniae* were the most difficult to treat but also the frequency of methicillin-resistant *S. aureus* appeared to be on the rise. In our hospital, surveillance and efforts to reduce multidrug resistant bacteria should be enforced, particularly focusing on these species, and in specific settings (i.e., intensive care units).

**Keywords**: antibiotic resistance, ESKAPE, Italy, hospital units, organs and systems.

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INTRODUCTION

Multi-resistant bacteria cause every year around 700,000 deaths worldwide and they are estimated to cause 10 million deaths by 2050 with a severe loss of economic resources [1,2]. Monitoring the epidemiology is one of the milestones for reducing mortality and morbidity of infections due to resistant bacteria. Indeed, it provides useful information for prevention and helps clinicians to empirically prescribe an effective antibiotic therapy [3]. Since 1998, the European Antimicrobial Resistance Surveillance Network, composed by 837 laboratories in 31 nations, is monitoring these infections. Fifty of these laboratories are in Italy, which in turn compose the Italian surveillance system (ISS) [4-6].

Calabria is a region located in the Southern part of the Italian peninsula and its territory is organized in 5 provinces with a population of 1,970,521 inhabitants. However, only the provinces of Reggio Calabria and Cosenza, which together comprise a population of 1,270,236 inhabitants, have a laboratory included in the ISS [6,7]. To our best knowledge, there are not published articles which evaluated multi-resistant bacteria in the Calabria region, except from two small works which evaluated prevalence of glycopeptide resistant enterococci in 2003 and relationships between the use of antimicrobial drugs and patterns of resistance of Streptococcus pneumoniae [8,9] and two local studies describing small epidemics of unusual pathogens [10] and successfull control [11].

Purpose of this study was to provide data about epidemiology of resistant bacteria in a teaching hospital (Mater Domini) of a province (Catanzaro) not included in the ISS and give a useful tool to improve both patient outcome and resource management by making the empirical antibiotic therapy more oriented. More specifically, this study describes the frequency of multi-resistant bacteria among patients admitted in different types of unit and with isolation of bacteria from different sites. Also, the frequency of isolation of multi-resistant bacteria over a four-year period (2011-2014) was evaluated.

MATERIALS AND METHODS

Data collection
We conducted a retrospective study, analyzing all first bacterial isolates from patients admitted at Mater Domini teaching hospital of Catanzaro, from January 1st, 2011 to December 31st, 2014. Mater Domini teaching hospital has 232 beds and it is one of the three public hospitals in the province of Catanzaro (363,057 inhabitants) [7]. Since we aimed at informing the choice of empiric antibiotic therapy at disease presentation, among bacterial species isolated from a single patient, only the first isolate was included in our analysis. When a second isolate of the same species was obtained, it was excluded from our analysis to mitigate any biases due to different clinical practices.

We focused our analysis on the ESKAPE group, which includes the most common bacteria with a profile of antibiotic resistance included as sentinel in the surveillance systems, modified as follows: Enterococcus faecium (or Enterococcus faecalis), Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa and Escherichia coli (as the main representative of the Enterobacteriaceae family) [12, 13]. Samples were gathered from different organs and systems: urine, blood, wound swabs, respiratory samples (sputum and broncho-alveolar aspiration fluid) and other samples (miscellaneous). Nasal and rectal swabs were excluded from our analysis because isolates could simply indicate colonization rather than infection, thus not needing in many cases any antibiotic treatments. Results of the study on swabbing and implication for surgical prophylaxis have been presented in a separate paper [14].

Samples were collected from patients admitted in 4 groups of hospital units: medical units, surgical units, cardiac intensive care unit (CICU) and intensive care unit (ICU). Pure bacteria cultures and antibiotic susceptibility tests were obtained through automated VITEK® system (bioMérieux). Susceptibility to antibiotics was evaluated through the breakpoints of European committee on antimicrobial susceptibility testing or EUCAST [15]. For Gram negative bacteria, five antibiotics were analyzed because they were routinely tested (ampicillin, as a beta-lactam of reference, and ciprofloxacin) or used for therapy of multi-resistant bacteria (colistin, meropenem and tygecicline). For A. baumannii, due to lack of clinical breakpoints for tygecicline, a minimal inhibitory concentration (MIC) ≤1 mg/l was arbitrary considered as the cut-off between susceptibility and resistance. Indeed, a MIC ≤1 mg/l has been proposed by some authors as a possible cut-off for
the choice of a tygecicline based treatment [16]. For Gram positive bacteria, the first choice beta-lactams (oxacillin for \textit{S. aureus} and ampicillin for Enterococci, if sensitive) were analyzed, plus among the most used drugs for treatment of multi-resistant bacteria: daptomycin, linezolid, tygecicline and vancomycin. To avoid the possible presence of hetero-vancomycin, intermediate resistant, \textit{S. aureus} (hVISA), a MIC ≤1 mg/l was considered as the cut-off between susceptibility and resistance.

\textbf{Gathering of isolates according to resistance profiles}
According to the standardized classification of the joined expert panel of the European Centre for Disease Prevention and Control (ECDC) and the US Centers for Disease Control and Prevention, we considered three groups of isolates with different antibiotic resistance patterns: group 1 (low resistant bacteria), including bacteria without resistant to any classes or with resistance to 1 molecule in no more than 2 classes; group 2 (highly resistant bacteria), including multi-drug resistant (MDR) bacteria with resistance to ≥1 molecules in ≥3 different classes and extreme-resistant bacteria (XDR) with resistance to ≥1 molecules in all but 2 or fewer classes; group 3 (pan-resistant bacteria, PDR) with resistance to all drugs and classes of antibiotics [17].

\textbf{Data analysis}
According to the recommendations of the guidelines on antimicrobial stewardship by the Infectious Diseases Society of America and the Society for Healthcare Epidemiology of America, we conducted a stratified analysis by various parameters (year, hospital unit, organs and system) [18].

We evaluated: i) number of isolates per bacterial species; ii) frequency of overall bacterial isolates included in group 1 or 2 or 3; iii) frequency of Gram negative isolates (\textit{E. coli}, \textit{K. pneumoniae}, \textit{A. baumannii} and \textit{P. aeruginosa}) included in group 2 or 3 which were resistant to ampicillin, ciprofloxacin, meropenem, tygecicline or colistin in 2014; iv) frequency of Gram positive isolates (\textit{S. aureus}, \textit{E. faecium} and \textit{E. faecalis}) which were resistant to the first-choice penicillins (oxacillin for \textit{S. aureus} and ampicillin for \textit{E. faecium} and \textit{E. faecalis}), tygecicline, linezolid, daptomycin (only for \textit{S. aureus}) or vancomycin in 2014; v) number of bacterial isolates per year; vi) frequency of bacterial isolates included in group 1 or 2 or 3 per year; vii) number of bacterial isolates by hospital unit; viii) frequency of bacterial isolates included in group 1 or 2 or 3 by hospital units; ix) number of bacterial isolates by organs and systems; x) frequency of bacterial isolates included in group 1 or 2 or 3 by organs and systems.

\textbf{Statistical analysis}
Statistical analysis for qualitative data was performed through $\chi^2$ test and significance was set at $p \leq 0.05$. Statistical analysis was performed to find differences between bacterial isolates with resistance to at least one molecule in <3 classes of antibiotics and bacterial isolates with resistance to at least one molecule in ≥3 classes of antibiotics (group 1 vs. group 2 plus 3) by hospital unit (medical units, surgical units, CICU and ICU) and by organs and systems (urine, blood, wound swabs, respiratory samples and other samples). Owing to the scanty frequencies, group 2 and group 3 have been pooled together in the statistical analyses. Similarly, data of CICU and ICU were pooled together because of the scanty frequency in CICU. Anyway, data for group 2 and 3 and for CICU and ICU were not pooled together in tables and figures for illustrative purposes. To assess the trend of resistance patterns (group 1 and group 2 plus 3) during the four years analyzed (2011, 2012, 2013 and 2014), $\chi^2$ test for trend (Cochrane-Armitage trend test) was applied. Use of $\chi^2$ test was considered to be not completely reliable (expected frequency below 1 or expected frequency less than 5 in more than 20% of cells as the condition for rely on the Gaussian approximation) in 44% of cases (15/27). Contingency tables of rows x columns from 2 by 3 to 2 by 7 were used.

\textbf{RESULTS}

\textbf{Bacterial species and patterns of resistance to antimicrobials}
During the four years under study, among the considered ESKAPE species (modified as previously described), 2,385 bacterial isolates were obtained. \textit{E. coli} was the most represented species with a frequency of 38\% (894/2,385), followed by \textit{P. aeruginosa} with 15\% (362/2,385), \textit{K. pneumoniae} with 14\% (341/2,385), \textit{S. aureus} with 13\% (316/2,385), \textit{A. baumannii} with 9\% (205/2,385), \textit{E. faecalis} with 8\% (192/2,385) and \textit{E. faecium} with 3\% (68/2,385). Distributions of bacterial species along calendar
years and by hospital units and types of samples are shown in Table 1. Overall, frequency of bacterial isolates with resistance to ≥1 molecules in <3 classes of antibiotics (group 1) and bacterial isolates with resistance to ≥1 molecules in ≥3 classes of antibiotics (group 2 plus 3) differed significantly by species (χ²=758.879; p <0.0001) (Figure 1). Also, in 2014, frequency of group 1 and group 2 plus 3 isolates were different (χ²=285.666; p <0.0001). The Gram-negative species with the highest frequency of isolates in group 2 plus 3 was A. baumannii (52/61=85%), followed by K. pneumoniae with 17% (13/78) and P. aeruginosa with 10% (8/78) (Figure 2). Only 1% (2/215) of isolates of E. coli were included in groups 2 plus 3, both resistant to ampicillin but susceptible to meropenem, tygecicline and colistin while one was resistant to ciprofloxacin. The Gram-positive species with the highest frequency of isolates included in group 2 plus 3 was S. aureus with 10% (9/91) (Figure 3). E. faecium was included in group 2 plus 3 in 8% (1/13) of cases, resistant to ampicillin but susceptible to linezolid, tygecicline and vancomycin. Only 2% (1/51) isolates of E. faecalis were included in group 2 plus 3 with conserved susceptibility to ampicillin, linezolid, tygecicline and vancomycin.

Resistance patterns by calendar years
Figure 4 shows number and percentage of bacterial isolates included in groups 1, 2 and 3 ranked by calendar years. From 2011 to 2014, changes in frequency of isolates with resistance to ≥1 molecules in <3 classes of antibiotics (group 1) and bacterial isolates with resistance to ≥1 molecules in ≥3 classes of antibiotics (group 2 plus 3) had a linear trend (χ²=55.093; p<0.0001). Indeed, frequency of isolates included in group 2 plus 3 decreased from 20% (129/648) in 2011 to 15% (88/587) in 2014.

Table 1 - Distribution of bacterial isolates among years, hospital units and samples.

<table>
<thead>
<tr>
<th>Category</th>
<th>Bacterial species</th>
<th>Total</th>
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<tbody>
<tr>
<td></td>
<td>E. faecalis</td>
<td>E. faecium</td>
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<tr>
<td></td>
<td>N</td>
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<tr>
<td>Year</td>
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</tr>
<tr>
<td>2011</td>
<td>39</td>
<td>19</td>
</tr>
<tr>
<td>2012</td>
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<td>2013</td>
<td>66</td>
<td>33</td>
</tr>
<tr>
<td>2014</td>
<td>51</td>
<td>26</td>
</tr>
<tr>
<td>TOT</td>
<td>199</td>
<td>100</td>
</tr>
<tr>
<td>Hospital Units</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medical units</td>
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<td>61</td>
</tr>
<tr>
<td>Surgical units</td>
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<td>2</td>
</tr>
<tr>
<td>ICU</td>
<td>23</td>
<td>12</td>
</tr>
<tr>
<td>TOT</td>
<td>199</td>
<td>100</td>
</tr>
<tr>
<td>Samples</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urine</td>
<td>70</td>
<td>35</td>
</tr>
<tr>
<td>Blood</td>
<td>34</td>
<td>18</td>
</tr>
<tr>
<td>Wounds wabs</td>
<td>57</td>
<td>28</td>
</tr>
<tr>
<td>Respiratory samples</td>
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<td>7</td>
</tr>
<tr>
<td>Other samples</td>
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<td>12</td>
</tr>
<tr>
<td>TOT</td>
<td>199</td>
<td>100</td>
</tr>
</tbody>
</table>
Figure 1 - Overall number and frequency of bacterial isolates.

Figure 2 - Frequency of antimicrobial drug resistance among MDR, XDR and PDR isolates (Gram negative bacteria, 2014). Figures 2a, 2b, and 2c show susceptibility rates to ampicillin, ciprofloxacin, colistin, meropenem and tygeclline among Acinetobacter baumannii, Klebsiella pneumoniae and Pseudomonas aeruginosa isolates, respectively, resistant to at least one molecule in ≥3 classes of antibiotics in 2014. Tygeclline was tested only in 38/52 A. baumannii isolates (read explanation in the text).
Patterns of multi-drug resistant bacteria

Analysis of single bacterial species by years showed that a linear trend toward an increase existed for *S. aureus* ($\chi^2=10.155; p=0.047$) while a linear trend toward a decrease existed for *E. coli* ($\chi^2=12.906; p=0.039$) and *K. pneumoniae* ($\chi^2=10.155; p=0.019$). For the remaining species, the trend was not statistically significant, although a tendency toward a decrease was noticed.

**Resistance patterns by hospital units**

Figure 5 shows number and percentage of bacterial isolates included in groups 1, 2 and 3 gathered
by hospital units. Frequency of isolates in group 1 and in group 2 plus 3 differed among hospital units ($\chi^2=204.599; p<0.0001$). In particular, frequency of bacterial isolates included in group 2 plus 3 was higher in ICU plus CICU (121/318=38%) than surgical units (103/630=16%) and medical units (99/1,437=7%). Analysis of single bacterial species ranked by hospital units showed that frequency of bacterial isolates in group 1 and group 2 plus 3 were different for K. pneumoniae (29/70=41% in ICU plus CICU, 27/78=35% in surgical units, 23/193=12% in medical units; $\chi^2=32.292$, $p<0.0001$), A. baumannii (71/87=81% in ICU plus CICU, 46/65=71% in surgical units, 29/53=55% in medical units; $\chi^2=6.947$, $p<0.0001$), and S. aureus (11/43=26% in ICU plus CICU, 8/132=6% in surgical units, 6/141=4% in medical units; $\chi^2=22.079$, $p<0.0001$).

Resistance patterns by sites of bacterial isolation (organ and system)
Figure 6 shows number and percentage of bacterial isolates included in groups 1, 2 and 3 gathered by different sites. Frequency of isolates with resistance to at least one molecule in group 1 and group 2 plus 3 differed among organs and systems ($\chi^2=192.341; p<0.01$). In particular, percentages of bacterial isolates included in group 2 plus 3 was higher in blood (88/274=32%) than other samples (47/204=23%), respiratory samples (82/374=22%), wound swabs (45/646=7%) and urine (52/887=6%). Analysis of single bacterial species by organs and systems showed that frequency of isolates with resistance to at least one molecule in <3 classes of antibiotics (group 1) and bacterial isolates with resistance to at least one molecule in ≥3 classes of antibiotics (group 2 plus 3) were different for E. faecalis ($\chi^2=33.735; p<0.0001$), E. faecium ($\chi^2=15.816; p=0.03$), K. pneumoniae ($\chi^2=21.181; p<0.0001$), P. aeruginosa ($\chi^2=27.819; p<0.0001$) and E. coli ($\chi^2=188.511; p<0.0001$).

**DISCUSSION**

This study, for the first time, provided data about resistance patterns of bacteria isolated from patients admitted from 2011 to 2014 to the Mater Do-
Patterns of multi-drug resistant bacteria

We conducted a stratified analysis to understand whether differences by different medical units and by source of samples exist, to identify the most urgent targets for infection control. Overall, Gram negative bacteria appeared to be the major causes of infection, either for frequency (E. coli) or patterns of resistance (A. baumannii and K. pneumoniae). One major explanation could be an over-representation of samples from anatomic sites where the Gram-negative flora is prevalent (especially urine). In absence of details on the clinical symptoms in patients whose samples were analyzed, this may also suggest the importance of avoiding urine collection from asymptomatic patients with urinary catheters. For Gram-positive bacteria, percentage of MRSA is increasing. Lastly, during the analyzed period, overall percentage of multi-resistant isolates reduced. It is not possible to explain this result but attention given to this problem with implementation of surveillance and preventative measures in our hospital may have played a role. However, this favorable trend in our hospital did not involve any species. In particular, percentages of multi-resistant isolates of both S. aureus and A. baumannii were higher in 2014 than 2011 (even though this increment was not statistically significant for A. baumannii). Anyway, efforts to reduce the spread of multi-resistant strains were somehow successful but efforts should be sustained.

According to gathering by hospital units, CICU and ICU had a significantly higher frequency of multi-resistant isolates than medical and surgical units, particularly for S. aureus, K. pneumoniae and A. baumannii. However, considering the relative low number of isolates from CICU, it is likely that, in our hospital the major “hotspot” of multi-resistant bacteria is ICU. In particular, the highest percentage of MRSA in ICU suggests that screening of patients and decontamination of MRSA carriers should be routinely performed. Importantly, the relative higher frequency of multi-resistant bacteria from blood rather than from other sites is relevant for empirical therapy in case of sepsis, indicating that drugs active against multi-resistant bacteria should be included at least in selected patients based on specific epidemiological and clinical factors.
Regarding individual species, *A. baumannii* retained the highest profile of antibiotic resistance. Particularly in 2014, 70% of isolates were included in group 2 (MDR and XDR), and 15% were included in group 3 (PDR). These data are consistent with data by Chelazzi et al. who found that 90.4% of *A. baumannii* was MDR in ICU [19]. However in our hospital resistance of *A. baumannii* to meropenem is even worse than demonstrated by European data [20]. Also, the high rates of resistance to tygecicline and colistin is of great concern, suggesting the utility to enforce more effective measures of antimicrobial stewardship to limit diffusion of these dangerous strains. For *K. pneumoniae* the rate of tygecicline resistance in 2014 (89%) was higher than in other healthcare institutions, such as that of Lombardi F. et al. (61%) [21]. Although percentage of isolates of *P. aeruginosa* with resistance to at least one molecule in ≥3 classes of antibiotics (groups 2 and 3) reduced, in 2014 percentage of multi-resistant isolates not susceptible to meropenem (50%) and fluoroquinolones (60%) was higher than in other settings, where *P. aeruginosa* was resistant in only 14% of cases to meropenem and 37% of cases to fluoroquinolones [20]. This fact suggests that a high percentage of patients admitted to our hospital would require a colistin based regimen to treat infections by *P. aeruginosa*. New antibiotics such as ceftazidime/avibactam and ceftolozane/tazobactam may provide a positive impact on emergence and spreading of carbapenemase producing strains [22-25].

Our data show that multi-resistant *S. aureus* is an emerging cause of morbidity in our hospital but differently from other settings where prevalence of hVISA among MRSA was higher (7% in India and 18% in Turkey), no MRSA isolate had a MIC >1 mg/l for vancomycin in 2014 [26, 27]. This fact suggests that in our hospital vancomycin-based regimens remain effective as empirical therapy in case of suspected infections due to MRSA.

Our study has several limitations. First, we do not know neither the clinical relevance of isolates (contaminants or pathogens) nor the outcome of patients from whom isolates were obtained and this limits the clinical implications of our work. Second, we did not investigate specific resistance mechanisms or enzymes such beta-lactamases and carbapenemases which may help tracking transmission routes for better infection control. Further studies are needed in this respect.

### CONCLUSIONS

In conclusion, among the Gram-negative bacteria, *A. baumannii* and *K. pneumoniae* appeared to be the two most difficult species to treat in our hospital, therefore efforts to reduce their incidence were needed. Also, more efforts were required to limit the hospital spreading of MRSA, which displayed an increasing trend from 2011 to 2014. We also showed heterogeneity in bacterial species and patterns of drug resistance among wards of the same hospital, which indicated that control measures should be individualized. Due to our results, some additional measures of containment of MDR infections and colonization have already been taken in our hospital. First, a new protocol of identification of MRSA carriers was started. Indeed, all patients in the cardiac surgery ward are screened for MRSA nasal colonization and, in cases of MRSA carriers, subsequently decontaminated. Secondly, a hospital protocol for surgical prophylaxis was written. This document was based on international guidelines of prophylaxis and modified with respect to our epidemiological data [28-30]. The effectiveness of such interventions appeared to be promising and will be monitored in future studies.

**Conflict of interest.**

The authors have no conflicts of interest to declare.

### REFERENCES


